polyvinyl alcohol, polysaccharides, polyvinyl ethyl ethers, α,β-Poly[(2-hydroxyethyl)-DL-aspartamide], RO-PEG, where R may be alkyl, aryl, alkyaryl, aroyl, alkanoyl, benzoyl, arylalkylethers, cycloalkyl, cycloalkylaryl, and derivatives of said polymers.

8. A compound according to Claim 7, wherein said water-soluble polymer is polyethylene glycol or a derivative thereof.

9. A compound according to Claim 8, wherein said water-soluble polymer is monomethoxypoly(ethylene glycol).

10. A water-soluble polymer modified polypeptide, said modified polypeptide produced by a method comprising the step,

mixing a compound according to Claim 1 with a polypeptide for modification.

11. A modified polypeptide according to Claim 10, wherein said polypeptide for modification is selected from the group consisting of hormones, lymphokines, cytokines, growth factors, enzymes, vaccine antigens, and antibodies.

12. A modified polypeptide according to Claim 11, wherein said polypeptide for modification is an antibody, said method further comprising the step of combining said antibody with a compound capable of specifically binding to a binding site on said antibody, prior to said mixing step.

13. A modified polypeptide according to Claim 11, wherein said polypeptide for modification is an enzyme, said method further comprising the step of combining said polypeptide with a substrate for said enzyme, prior to said mixing step.

14. A modified polypeptide according to Claim 11, wherein said polypeptide for modification is a glycoprotein.

15.A modified polypeptide according to Claim 14, wherein said glycoprotein is erythropoietin.

16. A modified polypeptide according to claim 9, said method further comprising the step of adding an oxidizing agent prior to said mixing step.

17. A composition comprising a polypeptide according to Claim 9 and a pharmaceutically acceptable carrier.

18. A composition comprising a polypeptide according to Claim 15 and a pharmaceutically acceptable carrier.

19. A compound belonging to the group consisting of,

a compound having the formula:

(X) $[P-O-CH_2-CO-NHN=CH-]_n-Z$,

a compound having the formula:

(XI) $[P-O-CO-NHN=CH-]_n-Z$,

a compound having the formula:

(XII) $[P-NH-CO-NHN=CH-]_n-Z$,

a compound having the formula:

(XIII) $[P-NH-CS-NHN=CH-]_n-Z$,

a compound having the formula:

(XIV) $[P-NHCO-NH-NHNHCO-NHN=CH-]_n-Z$,

a compound having the formula:

(XV) $[P-HNNHCON=CH-]_n-Z$,

a compound having the formula:

(XVI) $[P-HNNHCSN=CH-]_n-Z$,

a compound having the formula:

(XVII) $[P-NH-CO_6H_4-NHN=CH-]_n-Z$, and

a compound having the formula:

(XVIII) $[P-O-CO-CH_2CH_2-CO-NHN=CH-]_n-Z$

wherein Z is a polypeptide, n is 1 to x, x being the number of oxidation activatable groups on Z, and P is a water-soluble polymer.

20. A compound according to Claim 19, having the formula

(X) $[P-O-CO-NHN=CH-]_n-Z$.

21. The compound according to Claim 20 wherein n is 22-32.

22. A compound according to Claim 19, having the formula

(XI) $[P-NH-CO-NHN=CH-]_n-Z$

23. The compound according to Claim 22 wherein n is 17-25.

24. A compound according to Claim 19, having the formula

(XII) $[P\text{-}NH\text{-}CS\text{-}NHN{=}CH\text{-}]_n\text{-}Z$.

25. A compound according to Claim 19, having the formula

(XIII) $[P\text{-}NHCO\text{-}NH\text{-}NHNHCO\text{-}NHN{=}CH\text{-}]_n\text{-}Z$.

26. A compound according to Claim 19, having the formula

(XIV) $[P\text{-}HNNHCON{=}CH\text{-}]_n\text{-}Z$.

27. A compound according to Claim 19, having the formula

(XV) $[P\text{-}HNNCSN{=}CH\text{-}]_n\text{-}Z$.

28. A compound according to Claim 19, wherein P is selected from the group consisting of polyethylene glycol homopolymers, polypropylene glycol homopolymers, copolymers of ethylene glycol with propylene glycol, wherein said homopolymers and copolymers are unsubstituted or substituted at one end with an alkyl group, polyoxyethylated polyols, polyvinyl alcohol, polysaccharides, polyvinyl ethyl ethers, α,β-Poly[(2-hydroxyethyl)-DL-aspartamide], RO-PEG, where R may be alkyl, aryl, alkyaryl, aroyl, alkanoyl, benzoyl, arylalkylethers, cycloalkyl, cycloalkylaryl and derivatives of said polymers.

29. A compound according to Claim 19, wherein Z is selected from the group consisting of hormones,

lymphokines, cytokines, growth factors, enzymes, vaccine antigens, and antibodies.

30. A compound according to Claim 29, wherein Z is a glycoprotein.

31. A compound according to Claim 30, wherein said glycoprotein is erythropoietin.

32. A compound according to Claim 22, wherein Z is erythropoietin.

33. A compound according to claim 32 wherein said P is monomethoxypoly(ethylene glycol).

34. A compound according to Claim 33, wherein the average molecular weight of the monomethoxypoly (ethylene glycol) is in the range of 2000-12000.

35. A compound according to claim 34, wherein n is 10-36.

36. A compound according to claim 35, wherein n is 20-32.

37. A compound according to Claim 36, wherein the average molecular weight of the monomethoxypoly(ethylene glycol) is 5000.

38. A method of activating polypeptides for conjugation with compounds selected from the group consisting of compounds II, III, IV and V, said method comprising the step,

mixing a polypeptide for activation with an oxidizing agent.

39. A method according to Claim 38, wherein said oxidixing agent is sodium periodate.

40. A method according to Claim 39, wherein said periodate is present in a concentration of 10-40 micromoles per milligram of protein.

41. A method according to Claim 40, where said mixing step takes place at temperature in the range of -10-50°C

42. A method according to Claim 41, where said mixing step takes place at temperature in the range of 0-30°C.

43. A method according to Claim 42, where said mixing step takes for a period of time between 1 minute and 3 days.

44. A method according to Claim 43, where said mixing step takes place for a period of time between 1 minute and 60 minutes.

45. A method of making a water-soluble polymer modified polypeptide, said method comprising the step,

mixing a water-soluble polymer reagent compound according to Claim 1 with a polypeptide for modification.

46. A method according to Claim 44, wherein said polypeptide for modification is selected from the group consisting of hormones, lymphokines, cytokines, growth factors, enzymes, vaccine antigens, and antibodies.

47. A method according to Claim 10, wherein said polypeptide for modification is a glycoprotein.

48. A method according to Claim 46, wherein said glycoprotein is erythropoietin.

49. A modified polypeptide according to Claim 44, said method further comprising the step of adding an oxidizing agent to the polypetide for modification prior to said mixing step.

50. A method according to Claim 48, wherein said oxidizing agent is sodium periodate in a concentration in the range of 10-40 micromolar, P has a molecular weight in the range of 4000-12,000, said water soluble polymer is the compound of formula

(III) $P-NH-CO-NHNH_2$.

51. A kit for modifying polypeptides with water-soluble polymers, said kit comprising,

a water-soluble polymer according to claim 1.

52. A kit according to Claim 51, said kit further comprising, an oxidizing agent.

53. A kit according to Claim 48, said kit further comprising, a polypeptide for modification.

54. A compound having the formula:

P-Y-X-Q

wherein X is C=O, C= S, CH_2 or CHOH; Q is selected from the group consisting of $-ONH_2-$ and $-CH_2-ONH_2-$, and Y is selected from the group consisting of $-O-CH_2CH_2-$, $-O-CH_2CH_2-O-$, $-O-CH_2CH_2-N-$, $O-CH_2CH_2-S$, and $-O-CH_2CH_2CH-$; and P is a water soluble polymer.

55. A compound according to Claim 54, said compound belonging to the group consisting of,

a compound having the formula:

(XIX) $P-O-CH_2CH_2-CO-ONH_2$,

a compound having the formula:

(XX) $P-O-CH_2CH_2-O-CO-ONH_2$,

a compound having the formula:

(XXI) $P-O-CH_2CH_2-NH-CO-ONH_2$,

a compound having the formula:

(XXII) $P-O-CH_2CH_2-NH-CS-ONH_2$,

a compound having the formula:

(XXIII) $P-O-CH_2CH_2-ONH_2$,

a compound having the formula:

(XXIV) $P-O-CH_2CH_2-NH-CO-CH_2ONH_2$,

a compound having the formula:

(XXV) $P-O-CH_2CH_2-O-CO-CH_2-ONH_2$,

a compound having the formula:

(XXVI) $P-O-CH_2CH_2-CH(OH)-CH_2-ONH_2$,

a compound having the formula:

(XXVII) P-O-CH_2CH_2-CO-CH_2-ONH_2,

56. A compound according to Claim 55, said compound having the formula:

(XXI) P-O-CH_2CH_2-NH-CO-ONH_2,

57. A compound according to Claim 55, said compound having the formula:

(XXIII) P-O-CH_2CH_2-ONH_2,

58. A compound according to Claim 55, said compound having the formula:

(XXIV) P-O-CH_2CH_2-NH-CO-CH_2ONH_2,

59. A compound according to Claim 55, said compound having the formula:

(XIX) P-O-CH_2CH_2-CO-ONH_2.

60. A compound according to Claim 55, wherein said polymer is selected from the group consisting of polyethylene glycol homopolymers, polypropylene glycol homopolymers, copolymers of ethylene glycol with propylene glycol, wherein said homopolymers and copolymers are unsubstituted or substituted at one end with an alkyl group, polyoxyethylated polyols, polyvinyl alcohol, polysaccharides, polyvinyl ethyl ethers, α,β-Poly[(2-hydroxyethyl)-DL-aspartamide], RO-PEG, where R may be alkyl, aryl, alkyaryl, aroyl, alkanoyl, benzoyl, arylalkylethers, cycloalkyl, cycloalkylaryl, and derivatives of said polymers.

61. A compound according to Claim 60, wherein said water-soluble polymer is polyethylene glycol or a derivative thereof.

62. A compound according to Claim 61, wherein said water-soluble polymer is monomethoxypoly(ethylene glycol).

63. A water-soluble polymer modified polypeptide, said modified polypeptide produced by a method comprising the step,

mixing a compound according to Claim 61 with a polypeptide for modification.

64. A modified polypeptide according to Claim 63, wherein said polypeptide for modification is selected from the group consisting of hormones, lymphokines, cytokines, growth factors, enzymes, vaccine antigens, and antibodies.

65. A modified polypeptide according to Claim 64, wherein said polypeptide for modification is an antibody, said method further comprising the step of combining said antibody with a compound capable of specifically binding to a binding site on said antibody, prior to said mixing step.

66. A modified polypeptide according to Claim 64, wherein said polypeptide for modification is an enzyme, said method further comprising the step of combining said polypeptide with a substrate for said enzyme, prior to said mixing step.

67. A modified polypeptide according to Claim 64, wherein said polypeptide for modification is a glycoprotein.

68. A modified polypeptide according to Claim 67, wherein said glycoprotein is erythropoietin.

69. A modified polypeptide according to Claim 62, said method further comprising the step of adding an oxidizing agent prior to said mixing step.

70. A composition comprising a polypeptide according to Claim 62 and a pharmaceutically acceptable carrier.

71. A composition comprising a polypeptide according to Claim 68 and a pharmaceutically acceptable carrier.

72. A compound belonging to the group consisting of,

a compound having the formula:

(XXVIII) $[P\text{-}O\text{-}CH_2CH_2\text{-}CO\text{-}ON{=}CH\text{-}]_n\text{-}Z$;

a compound having the formula:

(XXIX) $[P\text{-}O\text{-}CH_2CH_2\text{-}O\text{-}CO\text{-}ON{=}CH\text{-}]_n\text{-}Z$;

a compound having the formula:

(XXX) $[P\text{-}O\text{-}CH_2CH_2\text{-}NH\text{-}CO\text{-}ON{=}CH\text{-}]_n\text{-}Z$;

a compound having the formula:

(XXXI) $[P\text{-}O\text{-}CH_2CH_2\text{-}NH\text{-}CS\text{-}ON{=}CH\text{-}]_n\text{-}Z$;

a compound having the formula:

(XXXII) $[P\text{-}O\text{-}CH_2CH_2\text{-}ON{=}CH\text{-}]_n\text{-}Z$;

a compound having the formula:

(XXXIII) $[P\text{-}O\text{-}CH_2CH_2\text{-}NH\text{-}CO\text{-}CH_2\text{-}ON{=}CH\text{-}]_n\text{-}Z$;

a compound having the formula:

(XXXIV) $[P-O-CH_2CH_2-O-CO-CH_2-ON=CH-]_n-Z$;

a compound having the formula:

(XXXV) $[P-O-CH_2CH_2-CH(OH)-CH_2-ON=CH-]_n-Z$; and

a compound having the formula:

(XXXVI) $[P-O-CH_2CH_2-CO-CH_2-ON=CH-]_n-Z$,

wherein Z is a polypeptide, n is 1 to x, x being the number of oxidation activatable groups on Z, and P is a water-soluble polymer.

73. A compound according to Claim 70, having the formula

(XXX) $[P-O-CH_2CH_2-NH-CO-ON=CH-]_n-Z$;

74. A compound according to Claim 72, having the formula

(XXXII) $[P-O-CH_2CH_2-ON=CH-]_n-Z$;

75. A compound according to Claim 72, having the formula

(XXXIII) $[P-O-CH_2CH_2-NH-CO-CH_2-ON=CH-]_n-Z$;

76. A compound according to Claim 72, having the formula

(XXVIII) $[P-O-CH_2CH_2-CO-ON=CH-]_n-Z$;

77. A compound according to Claim 72, wherein P is selected from the group consisting of polyethylene glycol homopolymers, polypropylene glycol homopolymers, copolymers of ethylene glycol with propylene glycol, wherein said homopolymers and

copolymers are unsubstituted or substituted at one end with an alkyl group, polyoxyethylated polyols, polyvinyl alcohol, polysaccharides, polyvinyl ethyl ethers, α,β-Poly[(2-hydroxyethyl)-DL-aspartamide], RO-PEG, where R may be alkyl, aryl, alkyaryl, aroyl, alkanoyl, benzoyl, arylalkylethers, cycloalkyl, cycloalkylaryl and derivatives of said polymers.

78. A compound according to Claim 72, wherein Z is selected from the group consisting of hormones, lymphokines, cytokines, growth factors, enzymes, vaccine antigens, and antibodies.

79. A compound according to Claim 74, wherein Z is a glycoprotein.

80. A compound according to Claim 75, wherein said glycoprotein is erythropoietin.

81. A compound according to Claim 76, wherein Z is erythropoietin.

82. A compound according to Claim 77 wherein said P is monomethoxypoly(ethylene glycol).

83. A compound according to Claim 78, wherein the average molecular weight of the monomethoxypoly(ethylene glycol) is in the range of 2000-12000.

84. A compound according to Claim 83, wherein n is 3-36.

85. A compound according to Claim 84, wherein n is 8-31.

86. A compound according to Claim 85, wherein the average molecular weight of the monomethoxypoly(ethylene glycol) is 5000.

87. A method of activating polypeptides for conjugation with compounds selected from the group consisting of compounds XIX, XXI, XXIII and XXIV said method comprising the step,

mixing a polypeptide for activation with an oxidizing agent.

88. A method according to Claim 87, wherein said oxidixing agent is sodium periodate.

89. A method according to Claim 88, wherein said periodate is present in a concentration of 10-40 micromoles per milligram of protein.

90. A method according to Claim 89, where said mixing step takes place at temperature in the range of -10-50°C

91. A method according to Claim 90, where said mixing step takes place at temperature in the range of 0-30°C.

92. A method according to Claim 91, where said mixing step takes for a period of time between 1 minute and 3 days.

93. A method according to Claim 92, where said mixing step takes place for a period of time between 1 minute and 60 minutes.

94. A method of making a water-soluble polymer modified polypeptide, said method comprising the step,

mixing a water-soluble polymer reagent compound according to Claim 52 with a polypeptide for modification.

95. A method according to Claim 94, wherein said polypeptide for modification is selected from the group consisting of hormones, lymphokines, cytokines, growth factors, enzymes, vaccine antigens, and antibodies.

96. A method according to Claim 94, wherein said polypeptide for modification is a glycoprotein.

97. A method according to Claim 96, wherein said glycoprotein is erythropoietin.

98. A modified polypeptide according to Claim 94, said method further comprising the step of adding an oxidizing agent to the polypetide for modification prior to said mixing step.

99. A method according to Claim 98, wherein said oxidizing agent is sodium periodate in a concentration in the range of 10-40 micromolar, P has a molecular weight in the range of 4000-12,000, said water soluble polymer is the compound of formula

(XXI) $P-O-CH_2CH_2-NH-CO-ONH_2$,

100. A kit for modifying polypeptides with water-soluble polymers, said kit comprising,

a water-soluble polymer according to Claim 54.

101. A kit according to Claim 100, said kit further comprising, an oxidizing agent.

102. A kit according to Claim 101, said kit further comprising, a polypeptide for modification.

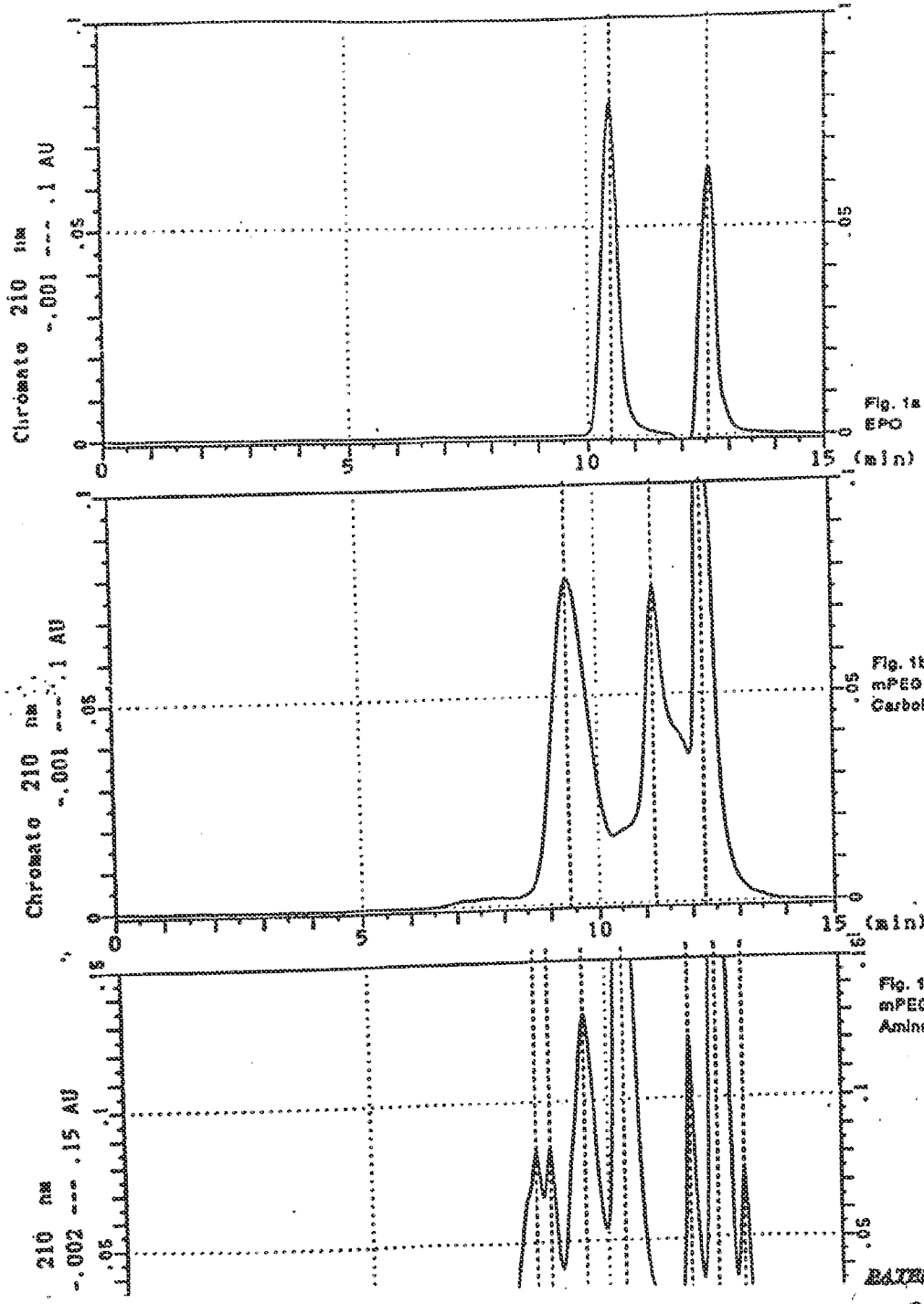

Fig. 1a
EPO

Fig. 1b
mPEG-EPO
Carbohydrate Modification

Fig. 1c
mPEG-EPO
Amino Acid Modification

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Figure 2: Hematocrit Levels in Mice
EPO and mPEG5000-EPOs,

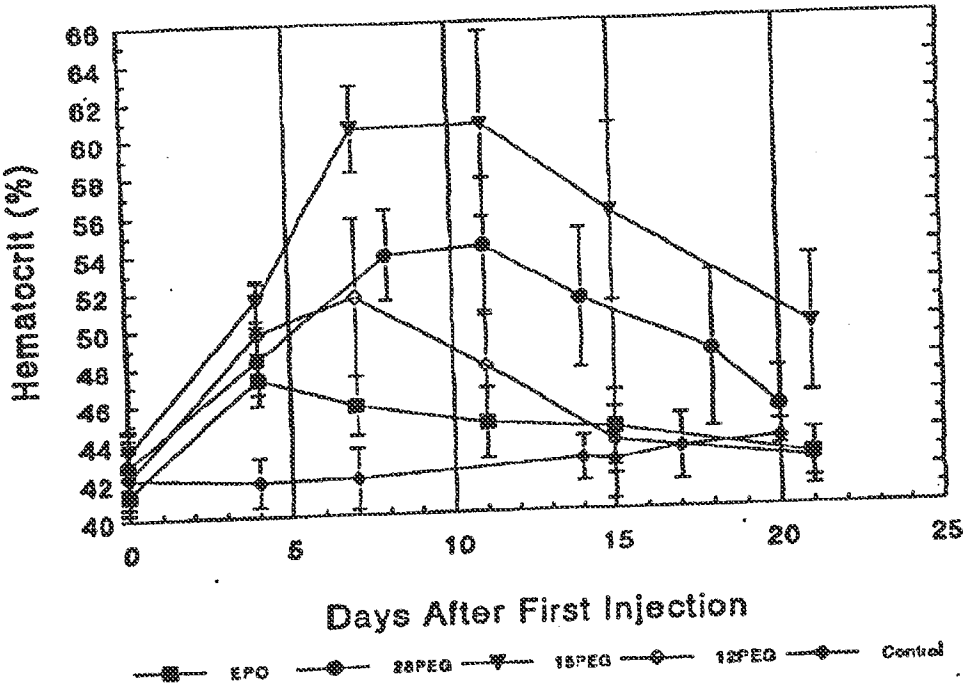

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Fig. 3

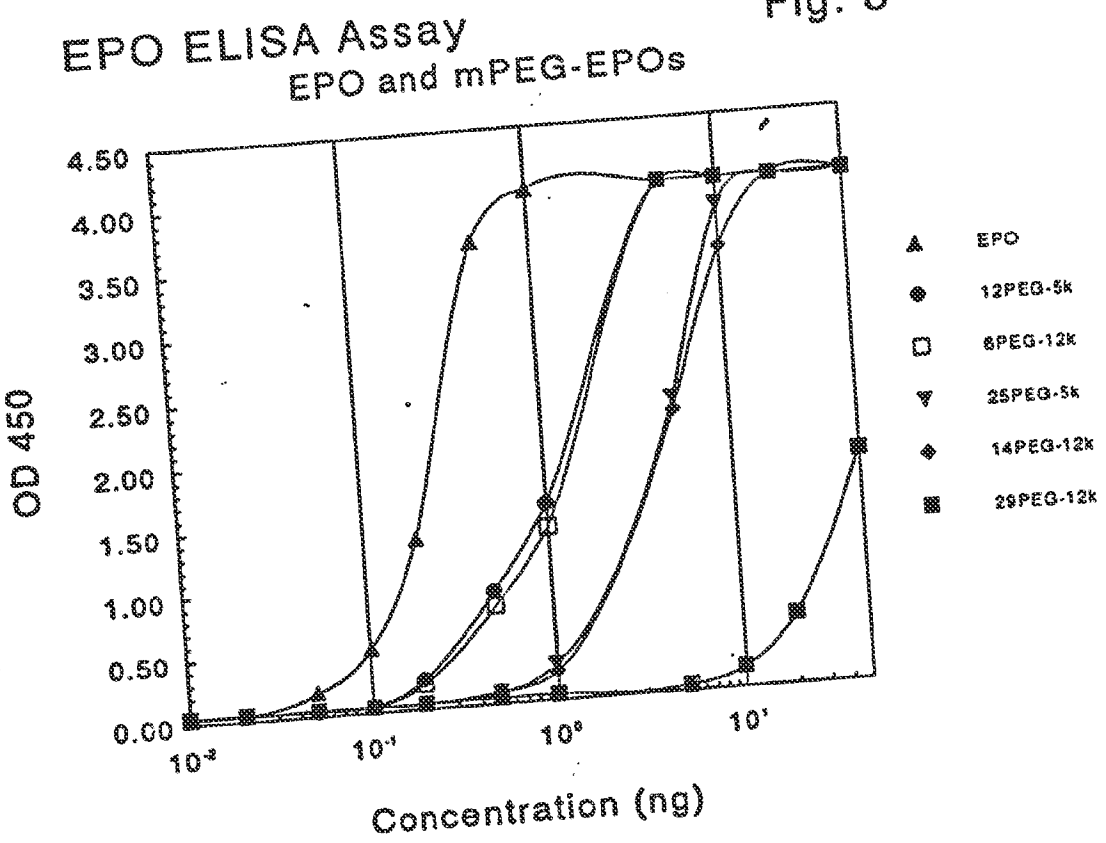

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Figure 4: Hematocrit Levels in Mice
Hydrazide and Semicarbazide Comparison

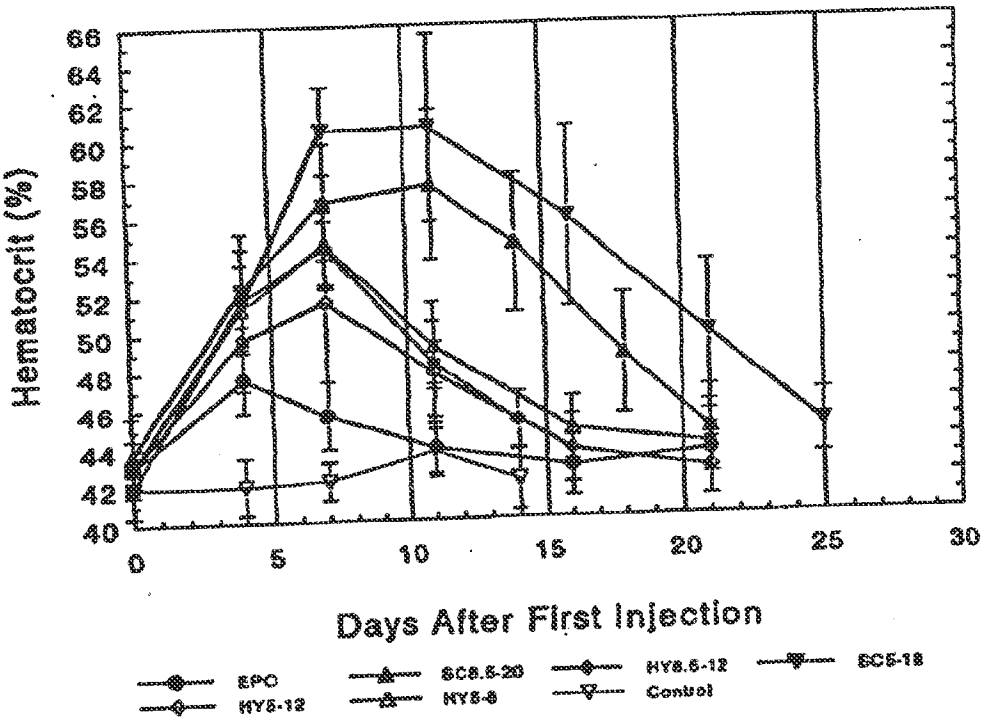

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Figure 5: Hematocrit Levels in Mice
EPO and mPEG8500-EPOs,

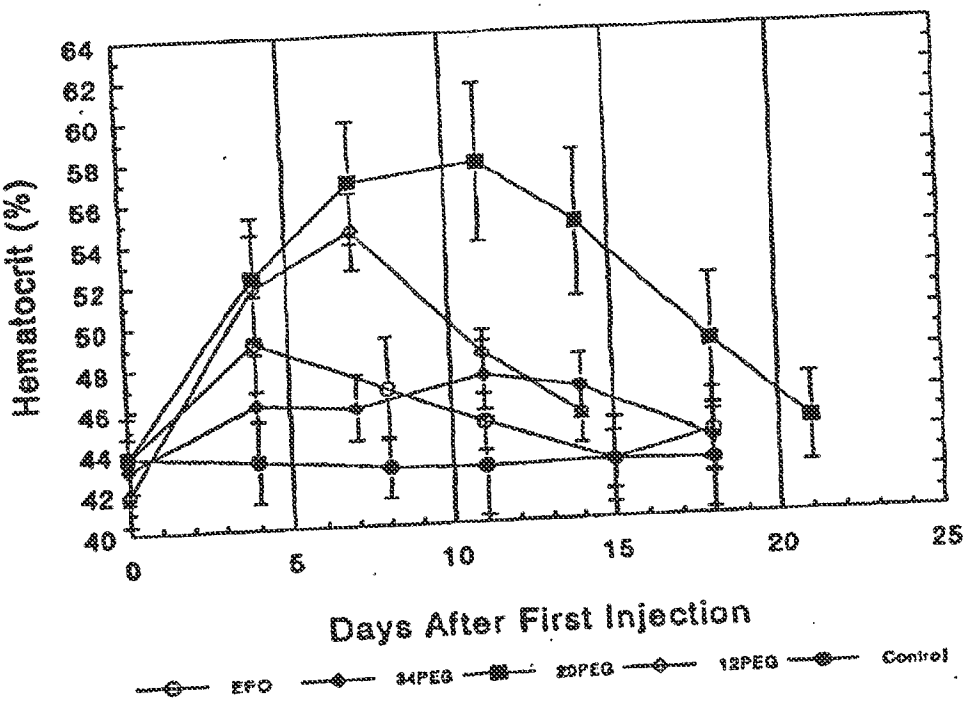

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FIGURE 6

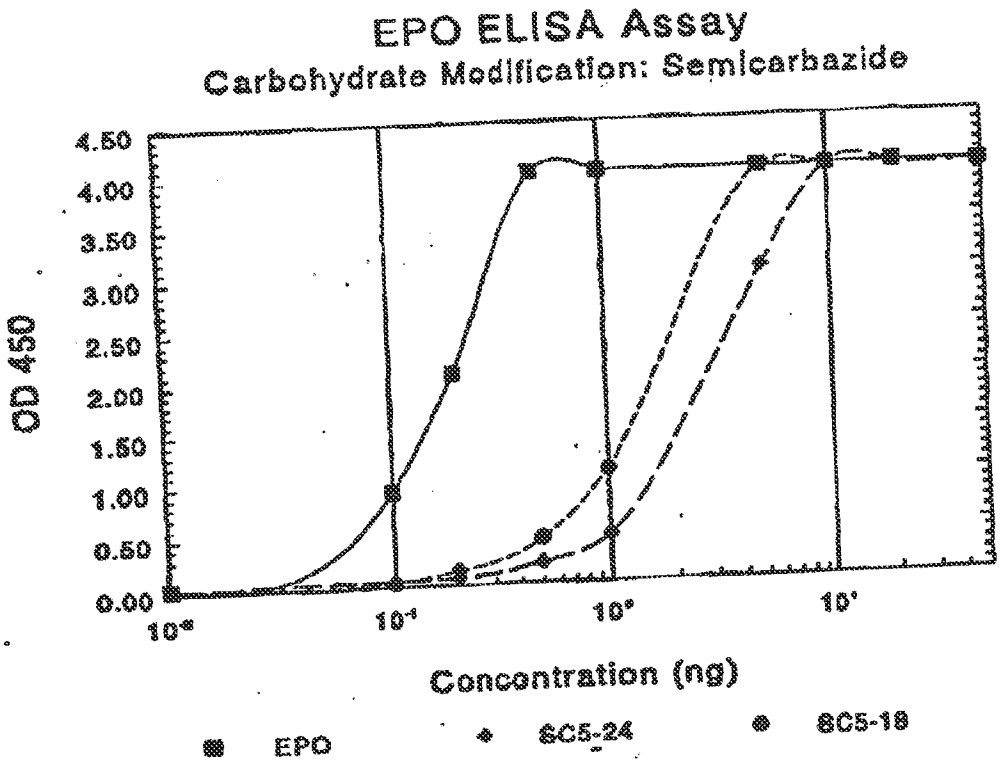

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FIGURE 7

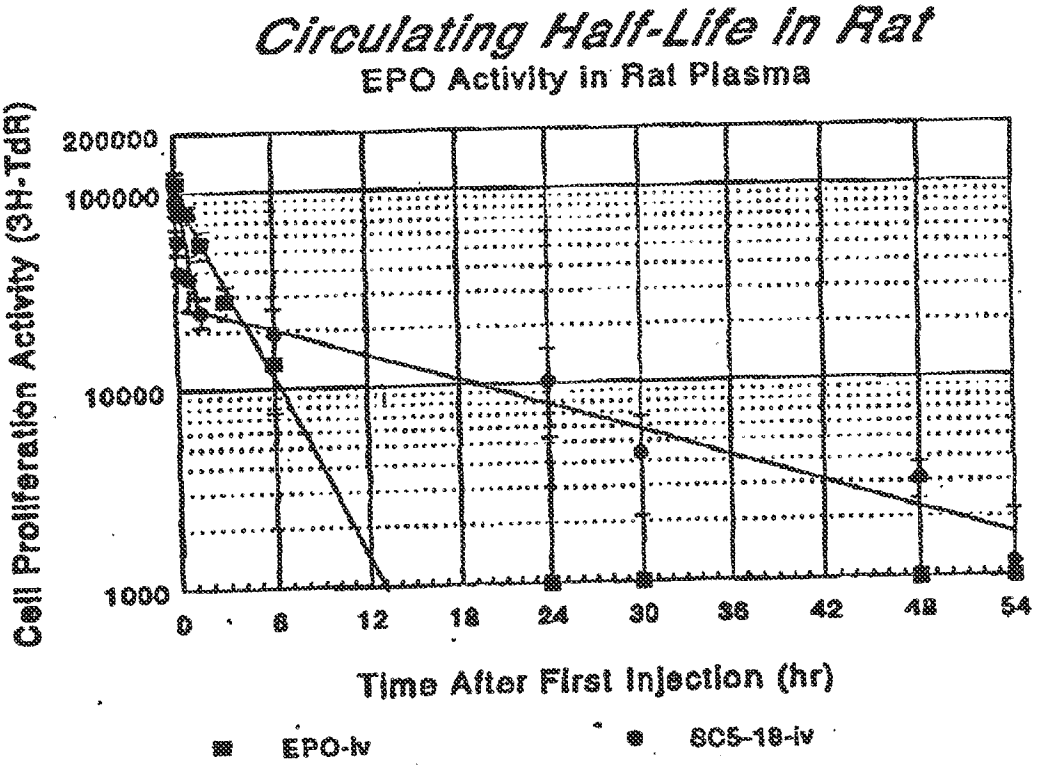

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FIGURE 8

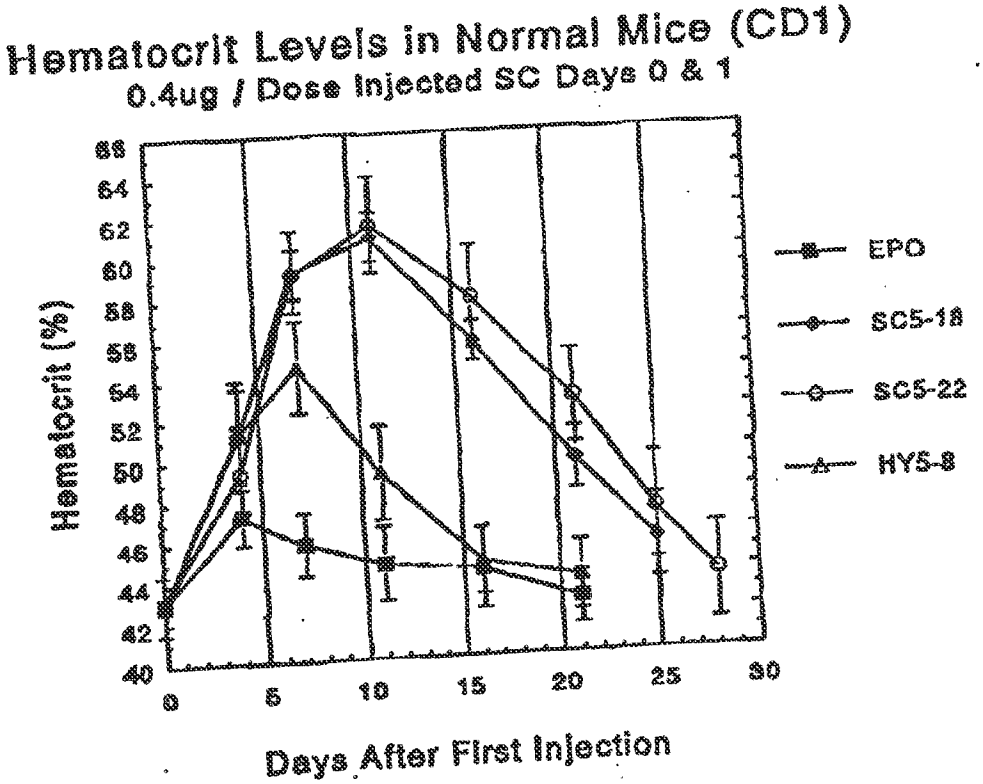

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FIGURE 9

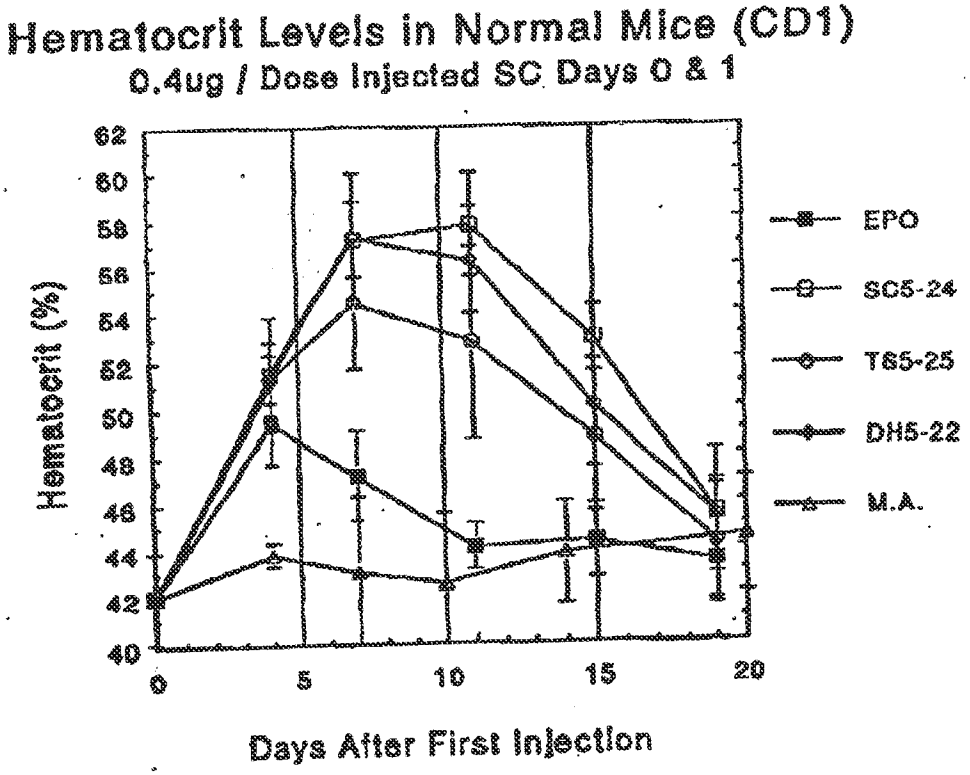

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FIGURE 10

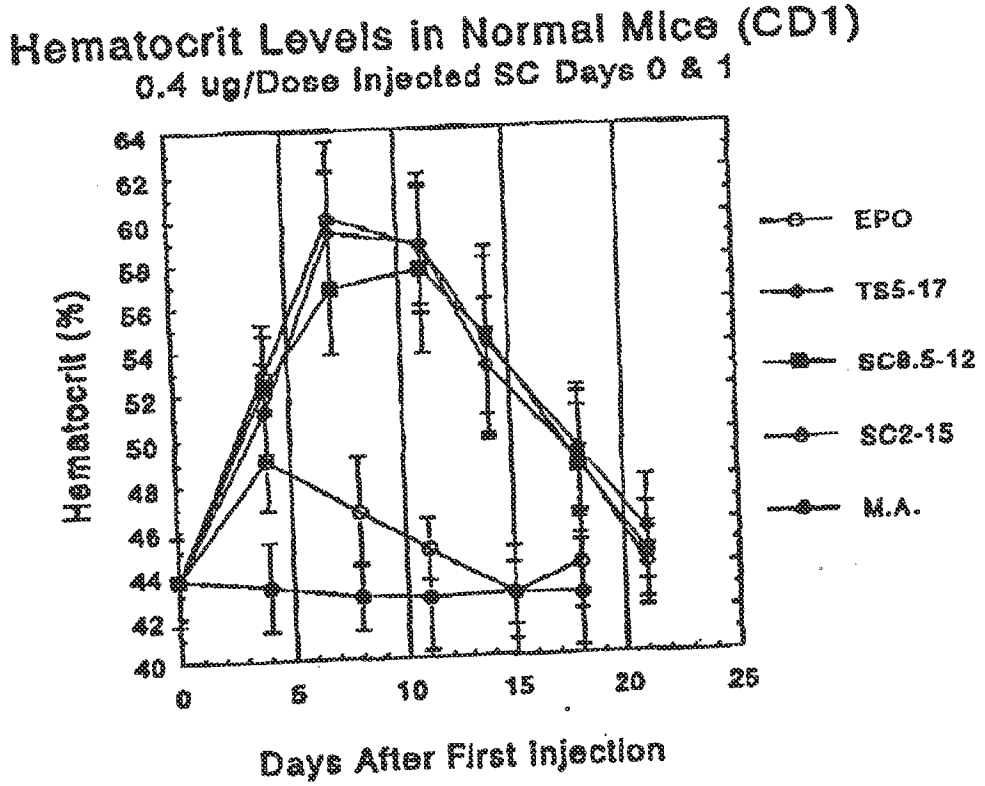

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FIGURE 11

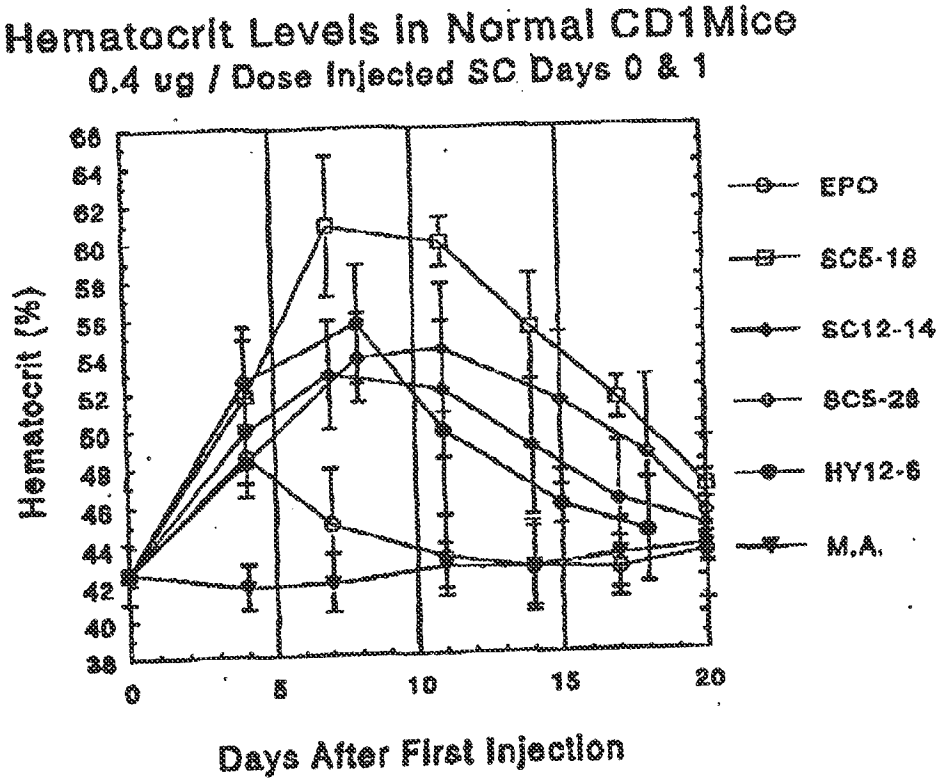

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FIGURE 12

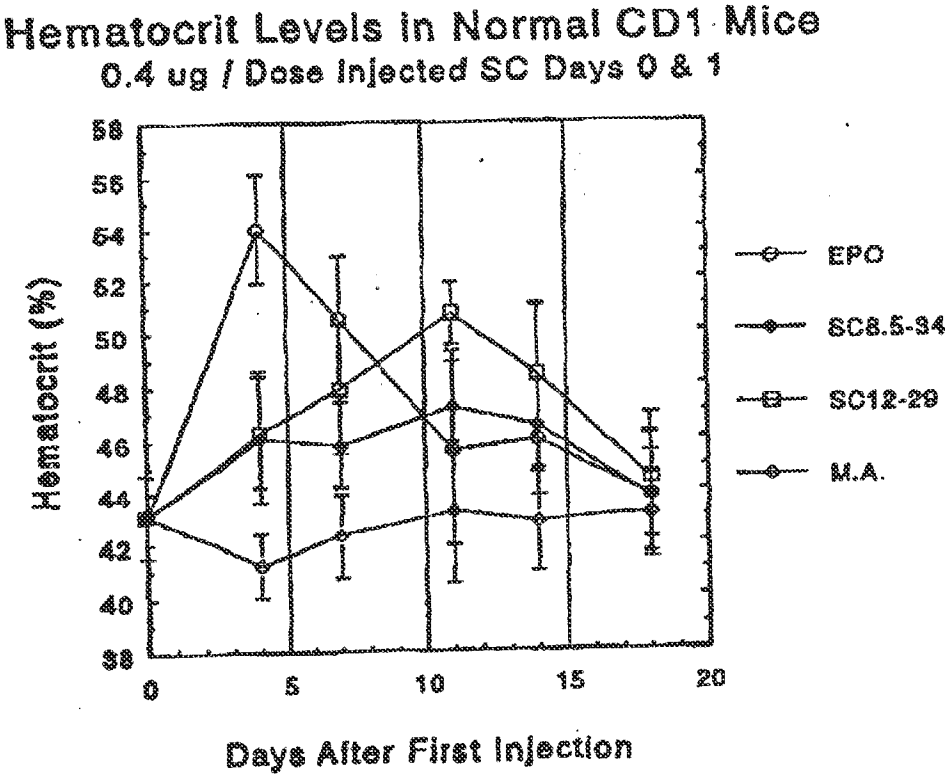

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FIGURE 13

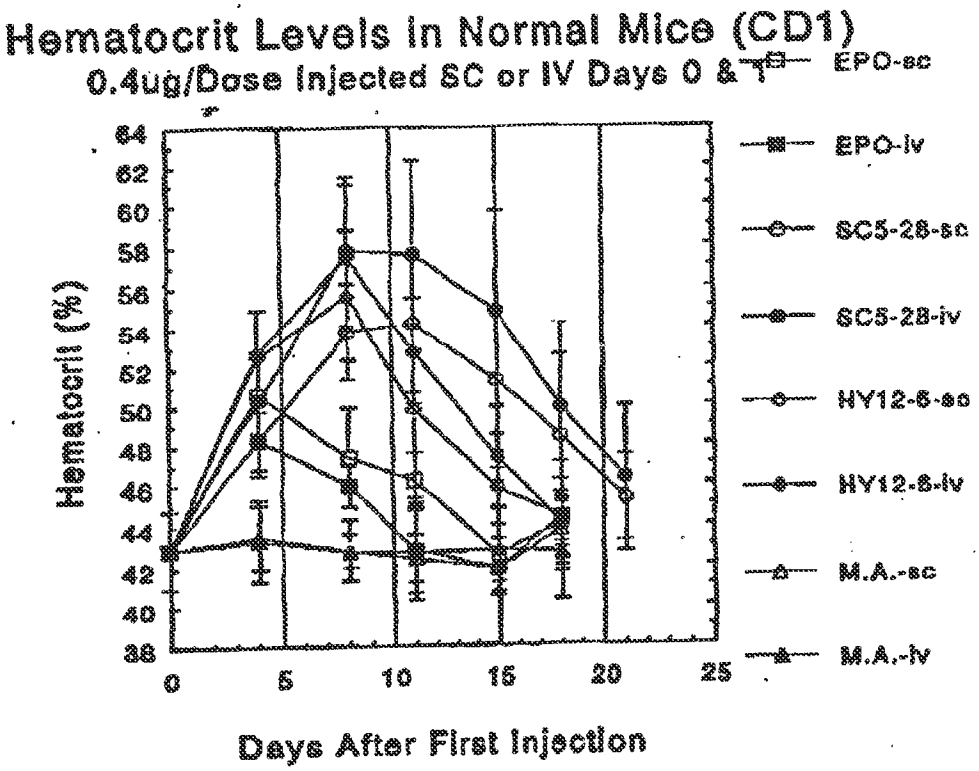

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FIGURE 14

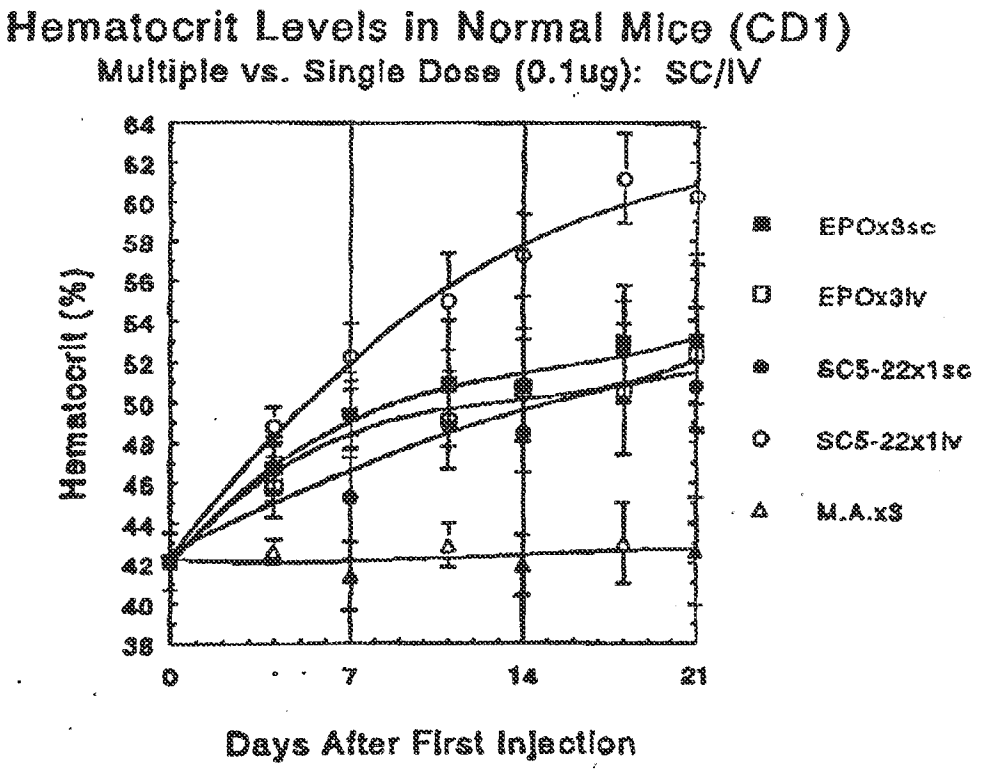

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FIGURE 15

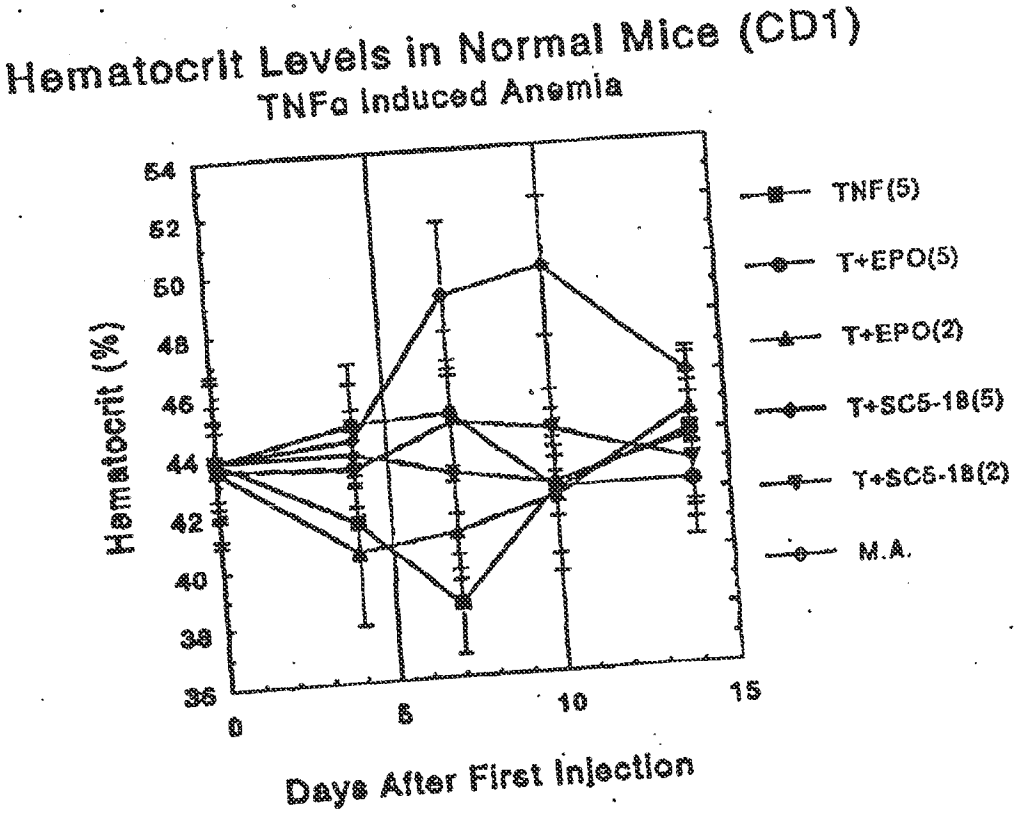

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Figure 16

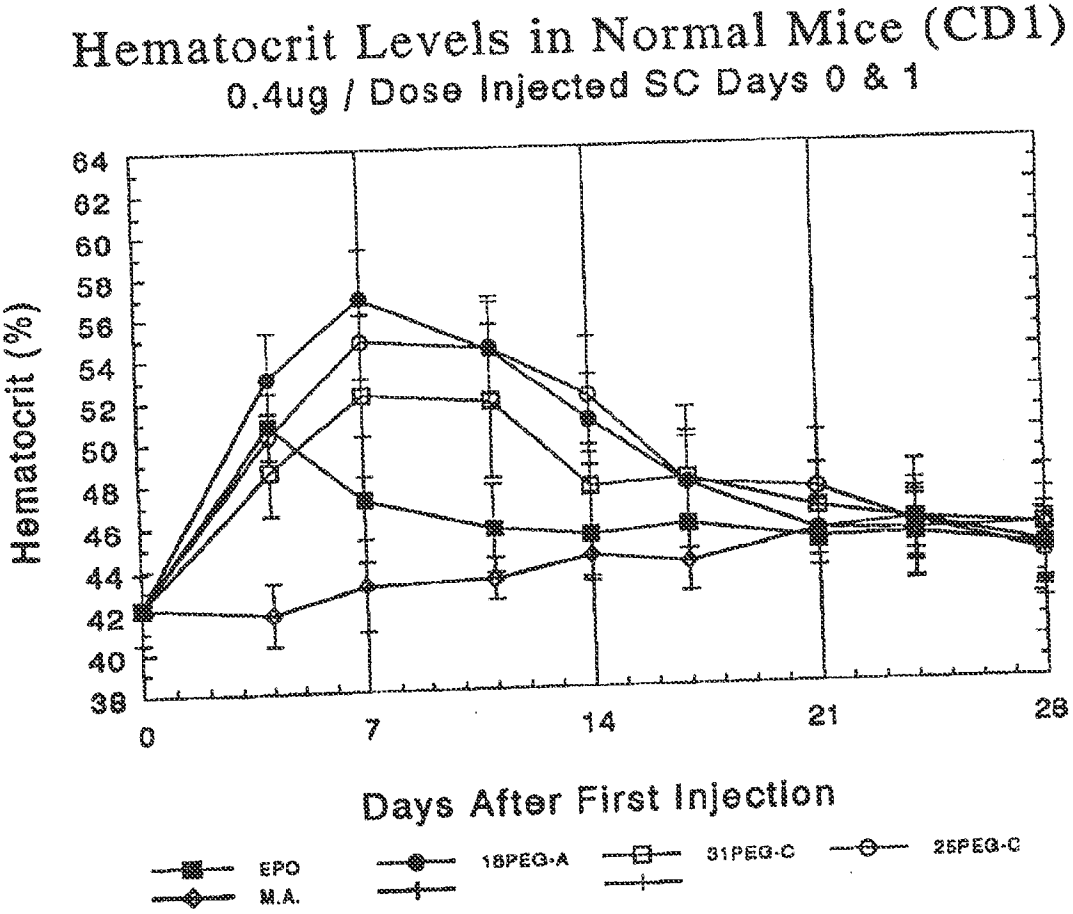

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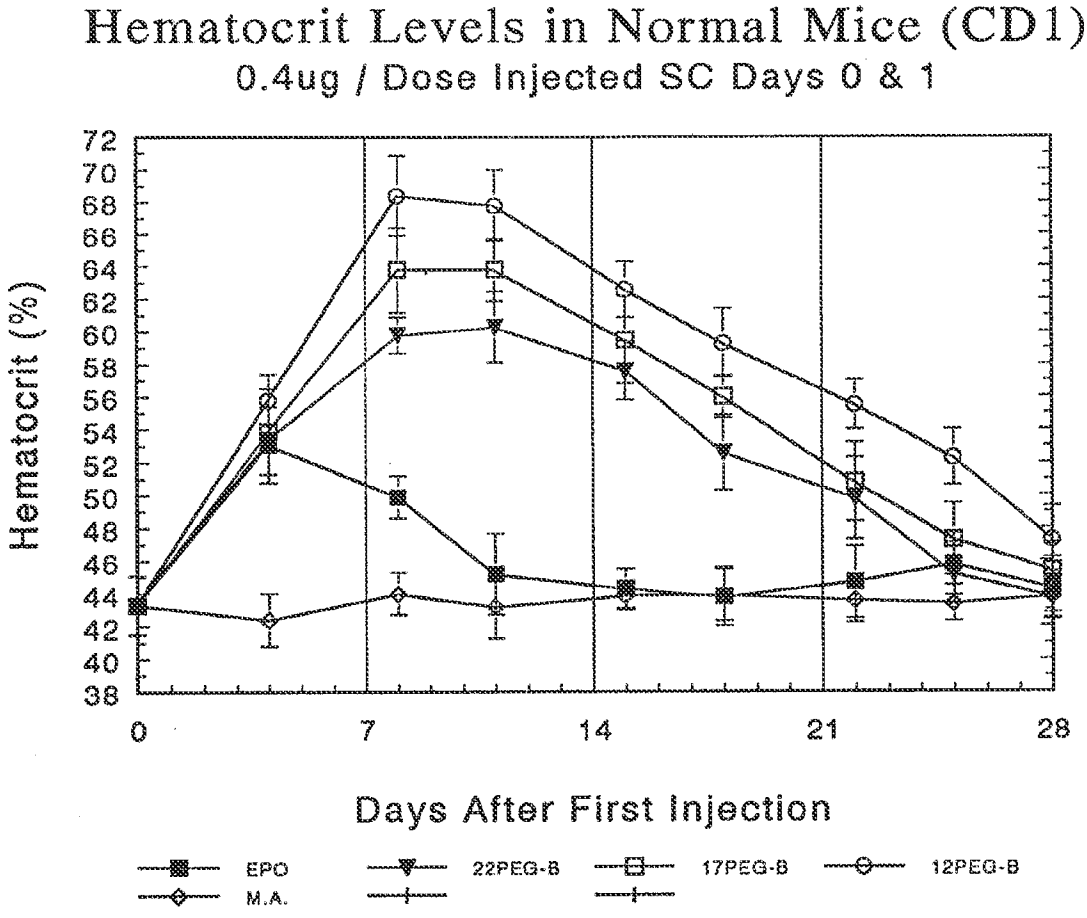
Figure 17



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Ridout Ogilvy Renault

Figure 18

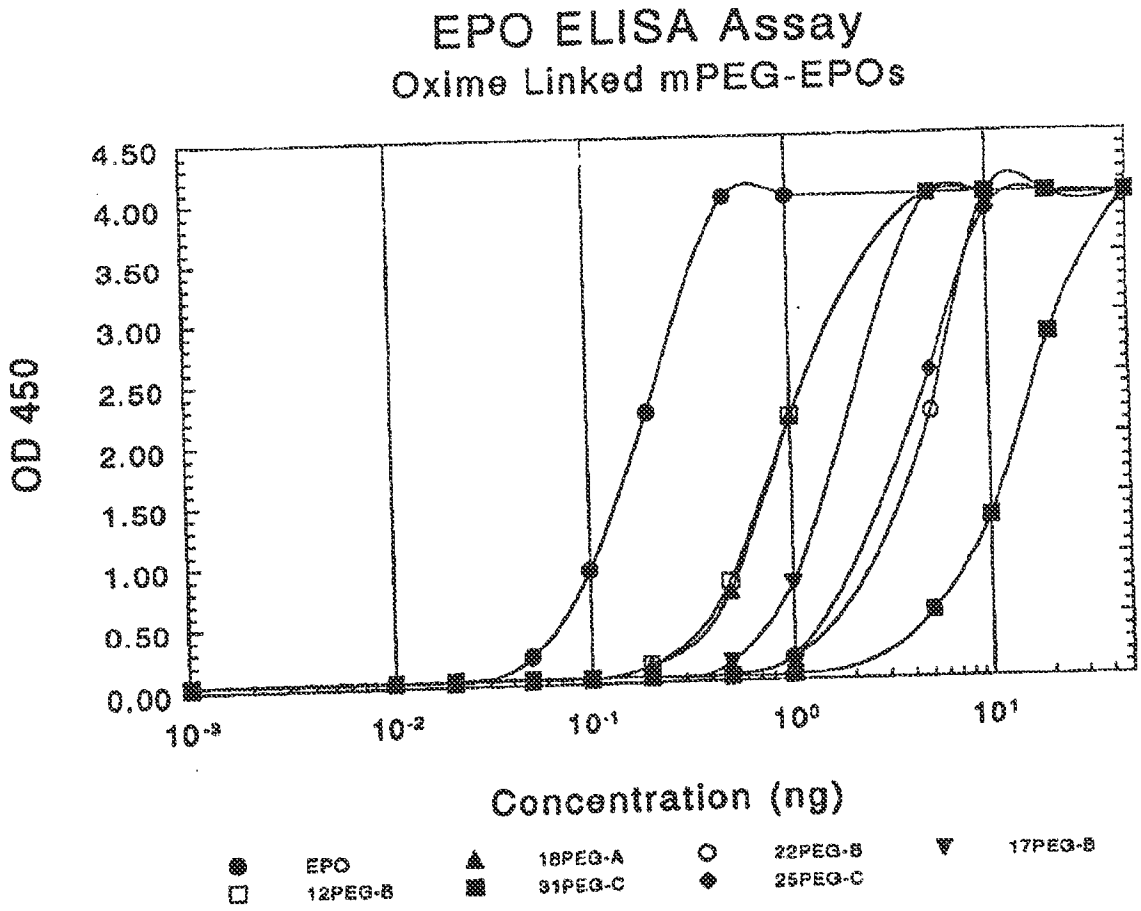

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Figure 19

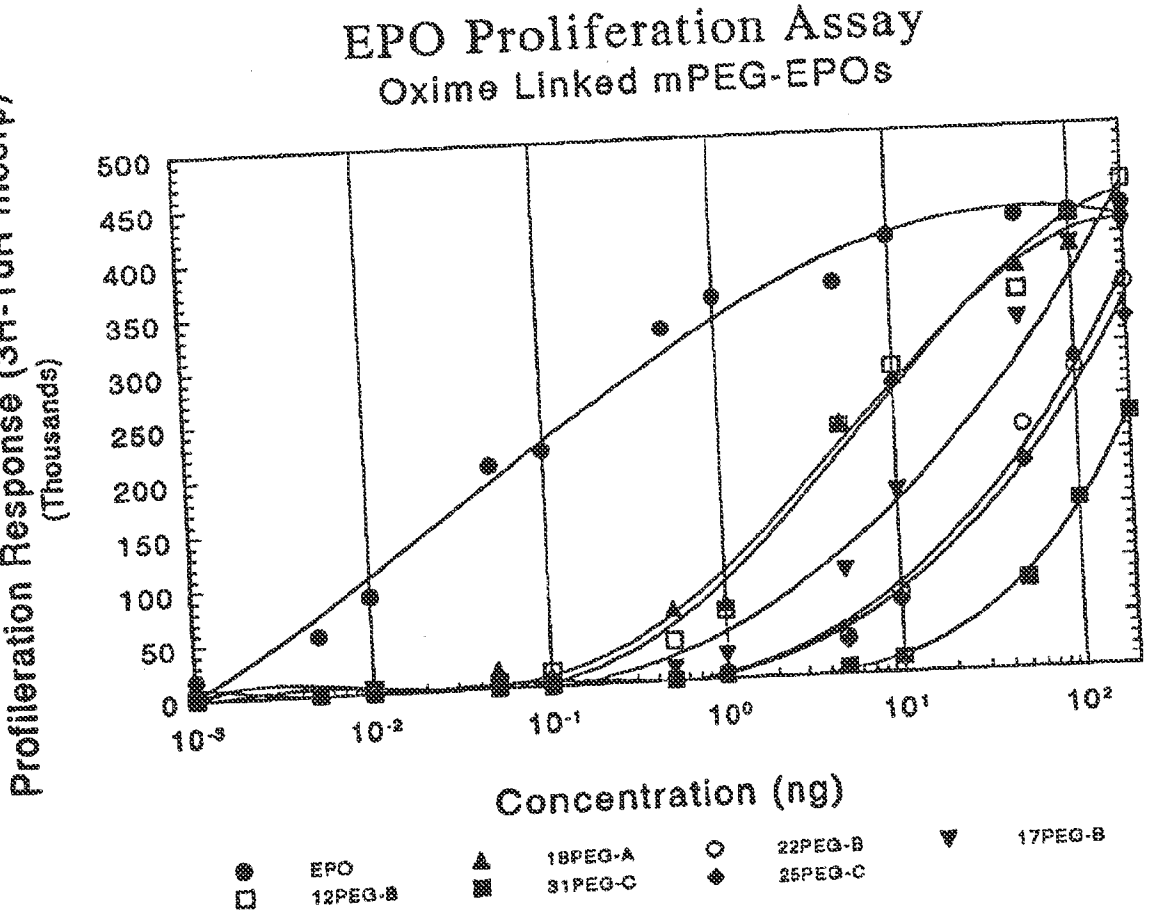

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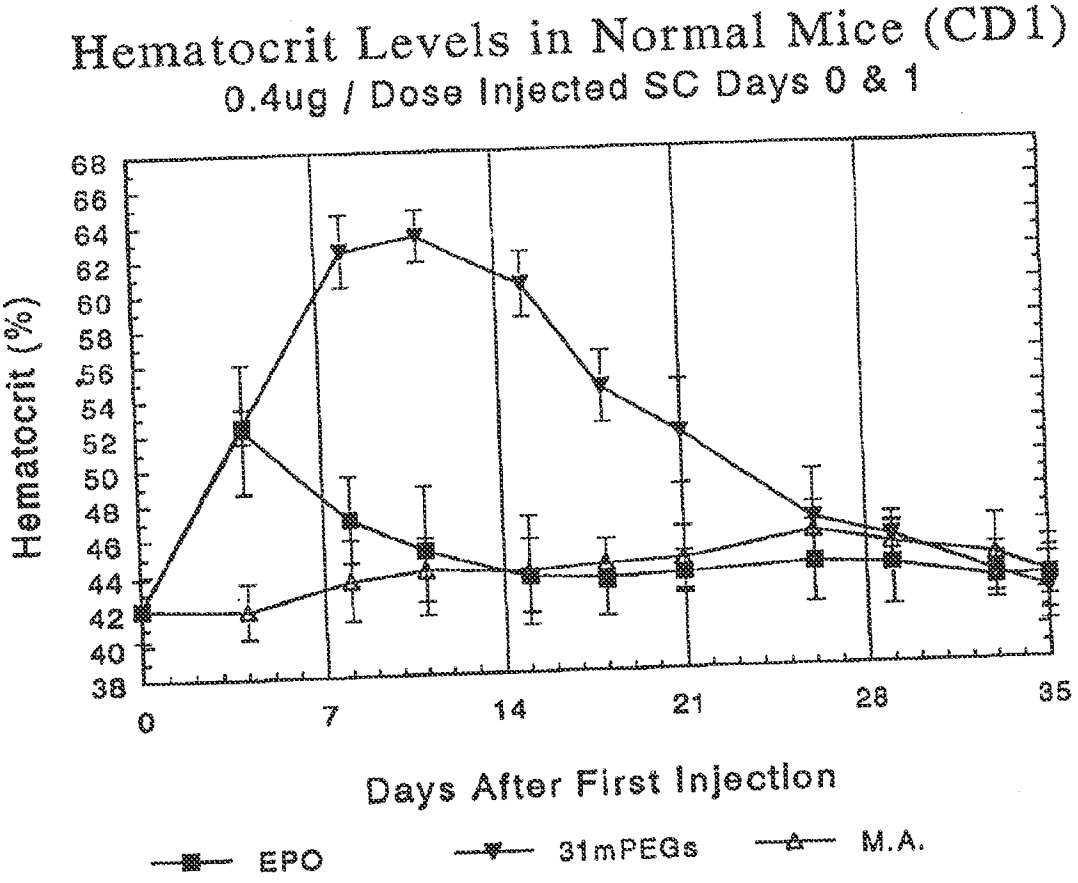
Figure 20



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